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II. Changes of ethanol contents in raw fish during storage

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and Y. Ota

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Usefulness of Ethanol as a Quality Index of Fish and Fish Products - II

Changes of Ethanol Contents in Raw Fish During Storage

Haruka IIDA, Toshio TOKUNAGA, Kooji NAKAMURA and Yoshiko OOTA

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Abstract: Recently, authors studied the relation between the quality of canned albacore and its content of ethanol, and reported that ethanol content seemed to be useful as an index of the quality of marine canned products.

So first, in this paper, the analytical method of ethanol in fish by gas-chromatography was examined. And then ethanol content of several species of fish during storage at 1—3 °C were measured and discussed in comparison with TMA-N content, volume of volatile components and pH. The sample fish used in this investigation were sardine, mackerel, yellow tail, alaska pollack, sand fish, horse mackerel and tuna. The results obtain were as follows;

1) The analytical method of ethanol used was as follows; An extract with sat. picric acid from fish meat was bubbled with synthetic air, and volatile components in this extract were led into a U tube that was immersed in liquid oxygen and trapped under the following conditions; air flow rate—80 ml/min., water bath temp.—70 °C and flow time—10 min.

Then the U tube was connected to GLC, and volatile components in the U tube were led to GLC and analyzed. The recovery of ethanol in this method was about 80%, and was enough for practical purposes.

2) Ethanol contents of raw fish increased during storage. And the ethanol contents of all fish species were 3—5 ppm except for sand fish, when the odor of spoilage was first perceived by sensory evaluation test.

3) Changes of TMA-N and total volume of volatile components were similar to that of ethanol contents. The pH of alaska pollack, sand fish and horse mackerel increased until about 7.0—8.0 area during storage. But the pH of other fishes was in the 5.5--6.5 area and there was little changes during storage.

4) When pH were below 6.7, the ratios of volatile TMA to total TMA content in fish were 0.2—0.5%, but ewhn the pH were above 6.7, the ratios were 1—5%.

5) Commercial can were analyzed, and much ethanol was measured in flake pack of canned yellow fin and small piece pack of canned pink salmon.

The authors analyzed the malodorous components of cans of boiled albacore yielding very different sensory evaluations, and reported having detected large volumes of ethanol in the bad cans[1]).

It has been known for a long time that when the freshness of fish and shellfish drops, ethanol is produced in the meat[2-7]). If the volume of ethanol production increases along a constant slope as the freshness drops, and if it is possible to detect ethanol quantitatively from heated sealed foodstuffs such as canned food, it seems to be possible to estimate the freshness of the raw materials used as well as the quality of the manufactured product by measuring the ethanol in the product.

Numerous physical and chemical characteristics have been proposed as indexes to determine the quality of the raw materials employed and that of the products. However, for heated products like canned food, the only existing methods of judging use as index components which are stable against heat such as nucleic acid-related substances[8]). So, we performed tests with the purpose of developing a simple method of evaluating the quality of the raw materials used for heated-sealed foostuffs and the quality of the product, a method using ethanol as an index. In this report, research is mainly based on the changes in the volumes of ethanol during the storage of the fresh fish and on comparisons between the volumes of trimethylamine (TMA), total volume of bad odor (volatile components) and the pH.

Method and results

1. Ethanol trapping method

After homogenizing the samples with equal volumes of saturated picric acid, we centrifuged for 10 min at 7,000 rpm, filtered the

supernatant with gauze and used the filtrate as sample solution. Using 2 mL of the sample solution, we trapped the volatile components with the same device as in our previous report[1]). However, as the ethanol recovery was insufficient under the previous conditions, we also examined the water bath temperature. Table 1 shows the recovery rates under various conditions. When raising the warm bath temperature and increasing the synthetic air flux, the volatilization volume of ethanol in the samples increased but so did also and simultaneously the volume of moisture evaporation in the sample solution. This evaporated moisture freezes in the U-type tube which becomes plugged. After various tests, the warm bath temperature was set at 70°C, the synthetic air flow at 80 mL/min and the traping time at 10 min. Under these conditions, the yield rate of the added ethanol was about 80%.

The GLC conditions are as follows. Apparatus: Shimadzu GC-4CM; packing: Porapak QS; column: 1.5 m x 3 mm glass column; column temperature: 150°C for 10 min; later, the temperature was raised to 200°C and the residues removed; injection port temperature: 170°C; carrier gas: N_2 30 mL/min; detector: FID; integrator: Shimadzu E-1A.

2. Method of measuring the odor volume

We measured, using the method explained in the former report[1]). That is, using 5 g of minced meat, we trapped the odor with a 40°C warm bath, a flow of 50 mL/min of synthetic air and 10 min of trapping time. At GLC analysis time, the column temperature was 150°C for 10 min. We raised it later to 190°C at the rate of 5°C per min and performed GLC for a total time of 90 min. The other conditions are the same as for the measurements of ethanol.

3. Measurement of TMA-N contents p. 85

We peformed GLC analysis according to the Tokunaga method[9]. The GLC conditions are as follows. Apparatus: Shimadzu GC-4BM; packing: chromosorb 103; column: 3 m x 3 mm glass column; column temperature: 140°C, raised to 200°C after TMA measurement; residual solvent removed; injection port temperature: 170°C; carrier gas: N_2 30 mL/min; detector: FID.

4. Samples

Table 2 shows the fish used as sample. Except for the tuna, the sample fish were used complete. They were stored at 1-3°C in a thermostatic container and taken out at regular intervals for use. We took the ordinary muscles of 3-6 sardines, sand fish, horse mackerel, cut them into small pieces and homogenized.

The tuna samples were blocks which had been carved out. We collected ordinary and dark muscles of mackerel, yellowtail, tuna to perform analysis. The sardine samples were brought frozen with dry ice immediately after being landed at Ibaraki prefecture fishing harbors. The other fish species were good quality fresh fish purchased at the Tsukiji market.

5. Changes in the various components during storage of the fresh fish

Figs. 1-7 show by fish species the changes in ethanol, TMA-N, pH and total odor volume during storage. The total odor volume is shown in integrator counts. The arrow sign under the axis of abscissas indicates the point at which a bad smell or drop in freshness began to be perceived in a sensory fashion. The solid lines mean ordinary muscle

and the broken lines dark muscle measurements. We showed under Figs. 1, 2 the symbols of the various items measured. These are common to the Figs. 1-7. We will explain the measured items separately.

pH

In the ordinary muscle of red fish such as sardine, mackerel, yellowtail, horse mackerel, tuna and the like, the pH was as low as 5.6-6.4 at the beginning of the tests. Except for horse mackerel, there was virtually no change during storage. Only the pH of horse mackerel rose from 6.4 to about 7.9 during storage. Meanwhile, the pH of the white fish Alaska pollack and sand fish is high. From the start of storage it was around 7.0 but climbed to about 7.5-8.0 during later storage, and conversely decreased from the time decomposition smell was produced. A comparison of the pH of the ordinary muscles and of the dark muscles shows that the pH was somewhat high at 6.0-6.4 in the dark muscles of mackerel and yellowtail. Meanwhile, the pH of the dark muscles of tuna was high at 6.6-7.7.

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Ethanol contents

The ethanol contents of the white fish Alaska pollack, sand fish increased suddenly from the 4th storage day. For the other red fish it started later, around the 6th day. The increase continued thereafter. The amount of ethanol at the time the smell of decay was noticed differs according to fish. In sardine, mackerel it was 13 ppm. In horse mackerel, tuna, Alaska pollack, sand fish it was respectively 20, 26, 32, 85 ppm. In most species of fish we noticed a sudden increase in ethanol volume from the moment (arrow sign in the graph) a drop in freshness could be smelled (bad odor) during sensory determination.

We noticed no great difference in ethanol contents between the dark and the ordinary muscles.

TMA-N contents

The TMA content variations showed the same variations as for ethanol. We mentioned previously that the moment at which ethanol increases suddenly coincide virtually with the time at which a drop in freshness is noticed in the sensory tests. As TMA and ethanol show very similar variations, it seems that TMA contributes greatly to the bad odor of dropping freshness. Furthermore, the TMA volumes of Alaska pollack, sand fish, horse mackerel are 30-100 mg/100 g at the time when the smell of decay appears and large when compared with the volumes in other fish species (5-20 mg/100 g).

When comparing the volume of TMA in dark and in ordinary muscle we see no noticeable difference just as in the case of the ethanol contents.

Total odor volume p. 88

The total odor volume too showed a striking similarity to the TMA-N contents. The total odor volume of Alaska pollack and sand fish rose sharply from the 2nd-4th day. It started to appear on the 6th-7th day in sardine, mackerel, yellowtail, tuna. The increase was somewhat less strong than in the two former species. In horse mackerel, the changes were halfway between the two groups. The above results indicate that the odor components are ethanol and TMA as well as aldehyde[10]) in Alaska pollack, sand fish, horse mackerel, and are ethanol and aldehyde in sardine, mackerel, yellowtail and tuna.

We noticed no difference in ethanol, TMA contents between dark and ordinary muscles. However, the odor volume was much greater in dark than in ordinary muscle. The reason is that the volume of fat is higher in dark than in ordinary muscle, and this is probably why a large amount of aldehydes such as acetoaldehydes, propionaldehydes, butylaldehydes and the like[11] is generated as fat oxidation products.

6. Relationship between the meat pH and the volume of TMA in the odor

Generally, the meat of white fish has a high pH after death. It is roughly neutral. As the freshness decreases, the pH rises. However, the pH in the meat of red fish is low after death, that is, often around 5.5-6.0 and it does not change thereafter. Furthermore, the TMA odor appears as freshness drops in white fish. In red fish, however, almost no TMA odor is perceived. We therefore examined the relationship between the proportion of TMA volatilized as odor in the total TMA on the one hand and the pH on the other.

With regard to TMA in the odor, we KOH-treated beads in a U-type tube used for trapping odor, connected to a GLC apparatus for TMA-N measurement and measured. So we were able to make a precise quantitative determination of TMA. Fig. 8 shows the results obtained by examining the pH and the TMA volatilization percentages.

When the pH of the meat is low, TMA does not volatilize readily in the form of gas. We saw that as the pH rises volatilization tends to proceed more easily, and it was ascertained that at a limit close to pH 6.7 the proportions of volatilizing TMA differ greatly. That is, when the pH is below 6.7, the proportion of TMA volatilized is only 0.2-0.5% but when the pH exceeds 6.7, the proportion is around 2%. It was at times higher than 5%.

7. Analytical results regarding commercial canned products

As a test, we analyzed the ethanol and nucleic acid-related substances in 2, 3 commercial canned products, using the aforementioned methods. To avoid any effect from seasonings, all canned products used were products kept in oil or boiled. We bought the samples at the supermarket purchasing price (August 1980).

Immediately after opening the can, we removed the juice by tilting, and analyzed the central meat used as sample. To measure the nucleic acid-related substances we used the enzyme method[8]. The results are shown in Table 3. Among the tuna can samples No. 2-5, the ethanol volume of the No. 4 yellowfin tuna flakes can was comparatively large, and the hypoxanthene (Hx) ratio was fairly high which suggests that the freshness of the original materials used for these cans was poor. In the No. 6-7 cans of boiled pink salmon, the ethanol volume was lower than in the canned tuna. Yet the ethanol volume in the No. 7 canned chunks was twice the volume found in the No. 6 canned ordinary muscle, and the Hx ratio was high too. One may say therefore that the materials used for can No. 7 were less fresh than those used for can No. 5. It is thus shown that the ethanol contents are significant as an index to determine at least the quality of canned fish boiled in water or preserved in oil.

Discussion p. 89

To preserve trace quantities of alcohol one may use the GLC method and the trace dispersion method[12]. However, below a certain volume of alcohol, one has to concentrate. If water is coexistent at concentrating time, there is concomitant boiling, and it is impossible by the usual methods to separate the water from the alcohol. If one

considers this point, the ethanol recovery rate (80%) of this method seems to represent a quite satisfactory result. As for the detection limit, full scale is precisely obtained on the chart when measuring 2 μg of ethanol by the present method. Therefore, measurements are possible if the ethanol contained in the samples is above 0.2 μg which means 10%. At actual measurement time, the peaks preceding (acetonitrile) and those following (propionaldehyde) the ethanol peak on the chart are not that large in samples whose freshness has not dropped too much. Therefore, analysis was sometimes possible at levels below 0.2 μg.

When we compared the amounts of ethanol in the meat of the various fish with the results of sensory rating of freshness (all fish except tuna), we could ascertain that there was a sensory perception of decreased freshness smell when ethanol exceeded 3-5 ppm in sardine, mackerel, Alaska pollack, yellowfin tuna, and 8-15 ppm in tuna and sand fish.

Tuna has to be stored for a long time before it produces an odor of decreasing freshness, and sensory determination was difficult. If we consider the overall determination results obtained by sensory evaluators, we see that the odor of decreasing freshness started on the 9th day. However, there were evaluators who detected a "somewhat oily smell" already on the 6th day, and the TMA volume also rose sharply on the 6th day. If we keep this in mind and suppose that the decreasing freshness odor appeared between the 6th and 9th day, one may accept as with the other fish a 3-5 ppm ethanol content as a standard for determining a drop in freshness. The same seems to hold for yellowtail.

However, sand fish having ethanol contents as high as 5.12 ppm at purchase time, exceeded already 10 ppm after 2 days. The volume of

TMA was as low as 1.5 mg/100 g on the 2nd day. Since there are also such cases, one should study further the above standard values.

Ethanol increases during storage but as the storage period is extended, the aldehydes increase momentarily to decrease later. The reason is that there is a limit to aldehyde production and that the aldehydes decrease through volatilization in the air or are converted to other substances so that their decrease is unclear. With regard to the cause of ethanol production during the storage of fresh fish, Lerke et al.[7]) point to the glycolytic action and to the action of amino acid microorganisms. Considering the above aldehyde tendency toward decrease, we could also think of a production caused by acetoaldehyde reduction.

Through a few examples, we showed above the analytical results obtained regarding commercial canned products. The volumes of ethanol shown are not necessarily constant throughout the transformation of the raw materials used to make the products, to the products themselves. The reason is that to make canned bonito, albacore and the like, one must go through the boiling, cooling and arrangement processes. Moreover, as pink salmon and mackerel are always vacuum-canned, there seems to be a strong possibility of ethanol dispersion during the process. Hillig[4]) reported that about half of the ethanol contained in the original materials disperses and is lost during boiling, but that as the ethanol contents are greatly different in fresh and in stale fish, the loss of ethanol during boiling is not a too big problem when comparing both volumes.

If we compare our results with those of Hillig[3-6]), we see that for the ethanol contents at every degree of freshness, the analytical results obtained by Hillig are about 10 times higher. It is

not clear whether this is due to differences in the samples or to differences in the measuring method. The difference with our method is that after removing the aldehydes, Hillig changes the alcohols to aldehydes to make measurements. The GLC Porapak QS packing used in our method separates the various components in the order of molecular weights, and as the ethanol peak appears between the acetoaldehyde and propionaldehyde, our method seems to measure correctly and exclusively the ethanol.

We intend to study the measurement conditions for the alcohol contents and to research the correlation between the ethanol contents in raw materials of different freshness and in the manufactured products on the one hand and the results of sensory determination on the other.

Table 1 Ethanol (EtOH) addition recovery test

(1) flux 80 mL/min 10 min
1 mL of water + 1 mL of saturated picric acid + 5 μg of EtOH

a 温度	b 測定 EtOH 量	c 回収率
70℃	3.905 μg	78.1%
75	3.960	79.2
80	4.305	86.9

(2) 温度 70℃, 流量 80 ml/min. 10 min.

f アジ抽出液	添加 EtOH 量 e	測定 EtOH 量 b	回収率 c
2 ml	...	1.87 μg	...
2	2.5 μg	3.86	79.8%
2	5.0	5.9	80.8

a temperature b volume of EtOH measured c recovery rate
d flux e volume of EtOH added
f horse mackerel extract

Table 2 Sample fish

	T.L.	B.W.	number of samples
sardine	17 cm	40 g	3 each time
mackerel	39	586	1
yellowtail	47	1,086	1
Alaska pollack	46	741	1
sand fish	17	39	6
horse mackerel	18	54	4
tuna	...	...	carved up

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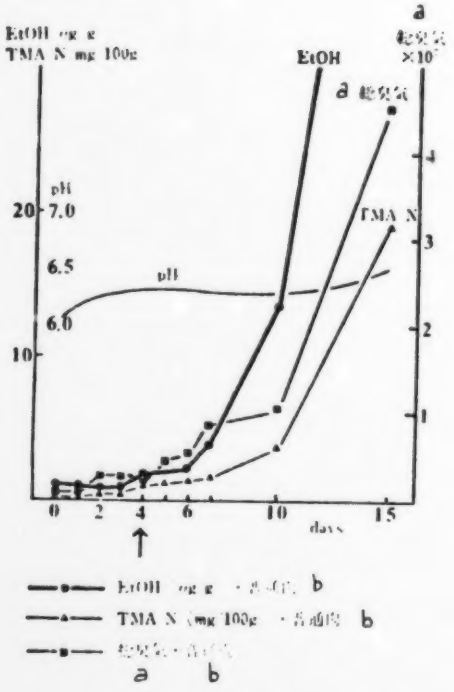

Fig. 1 Time-course changes in sardine

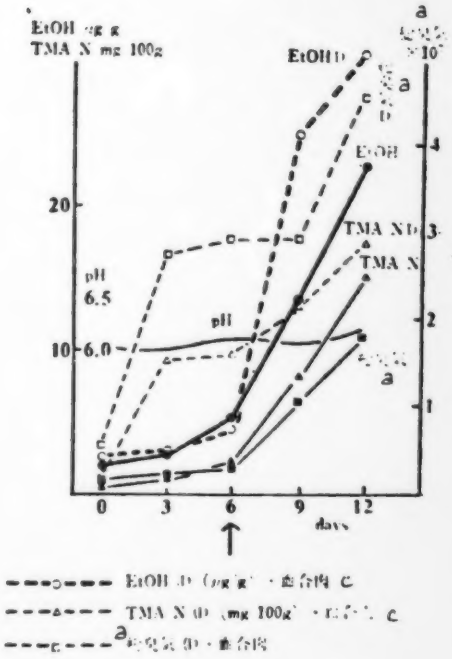

Fig. 2 Time-course changes in mackerel

a total odor
b ordinary muscle
c dark muscle

(same symbols in Figures 1 - 7)

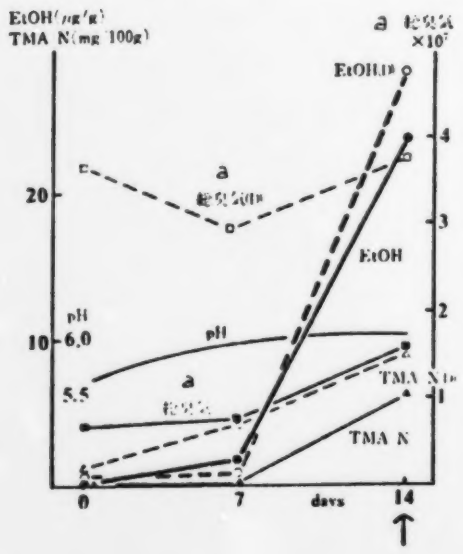

Fig. 3 Time-course changes in yellowtail

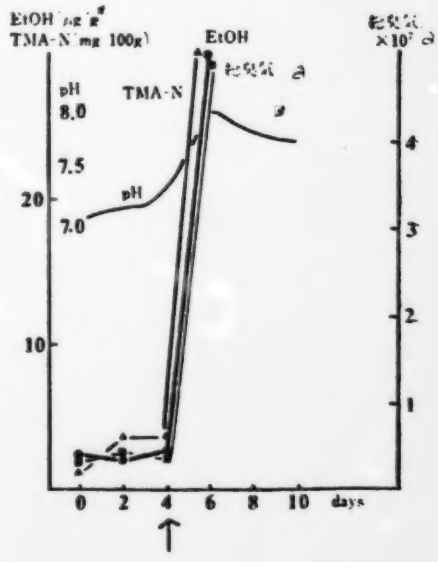

Fig. 4 Time-course changes in Alaska pollack

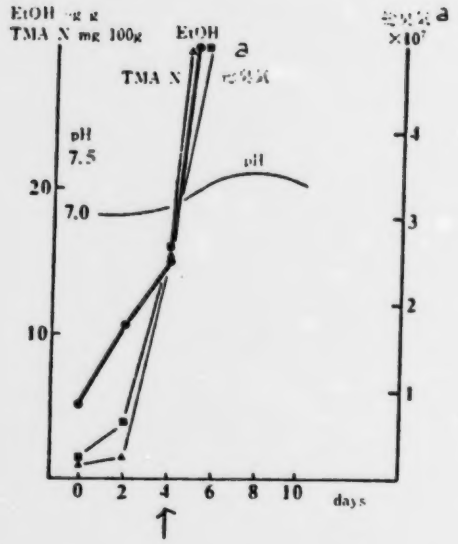

Fig. 5 Time-course changes in sand fish

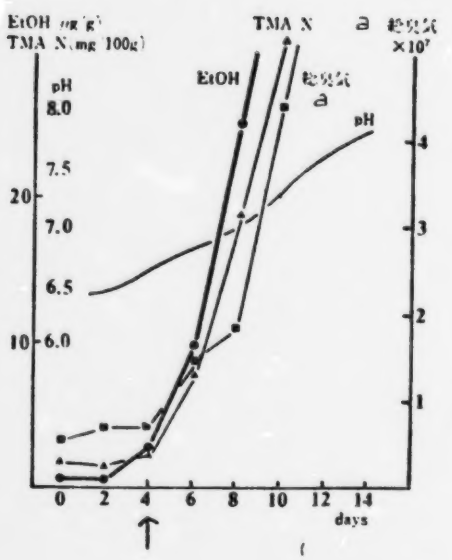

Fig. 6 Time-course changes in horse mackerel

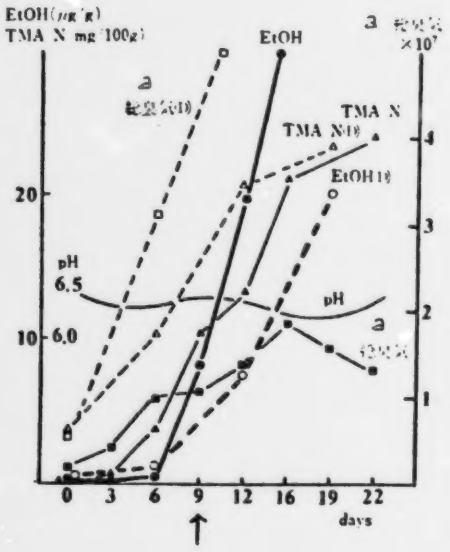

Fig. 7 Time-course changes in tuna

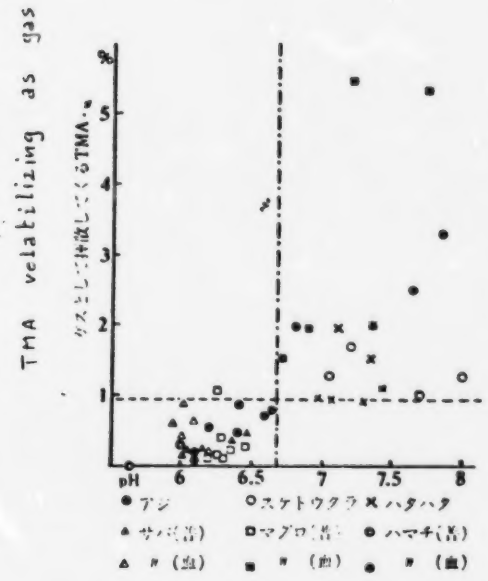

Fig. 8 Correlation between pH and amine volumes in odor
(% vs. contents)

● horse mackerel
▲ mackerel (ordinary)
△ mackerel (dark)
○ Alaska pollack
□ tuna (ordinary)
■ tuna (dark)
× sand fish
◎ yellowtail (ordinary)
⊘ yellowtail (dark)

Table 3 Results of the analysis of commercial canned foodstuff

a 試料	b 缶形(内容固形量)	c 価格	EtOH (μg/g)	Hx(%)	HxR(%)	IMP(%)	pH
1. カツオ油漬	e ツナ3号(85g)	177円	0.24	6.0	40.4	53.6	5.98
2. ビンナガ〃	〃 3号(85)	198 c'	2.48	16.8	51.0	32.2	6.02
3. 〃 〃	〃 2号(155)	278	2.77	5.9	29.9	64.2	5.95
4. ホハクフレーク〃	〃 2号(185)	190	4.40	36.3	31.6	32.1	6.40
5. ビンナカフレーク〃	〃 2号(185)	285	0.33	8.0	32.8	59.1	5.95
6. カラフトマス水煮	f 平 2号(220)	297	0.61	15.8	56.1	28.2	6.40
7. 〃 〃(細肉)	〃 2号(220)	215	1.37	37.0	49.1	13.9	6.60
8. サバ水煮	〃 2号(220)	100	0.89	6.1	46.2	47.6	6.22

a sample
b type of can (volume of solid matter contained)
c price c' yen
d No.
e tsuna (?)
f flat

1. bonito preserved in oil
2. albacore " " "
4. yellowfin tuna flakes
5. albacore flakes
6. pink salmon boiled in water
7. " " (chunks)
8. mackerel boiled in water

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